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# Diagnosis of Bacterial Vaginosis by Direct Gram Stain of Vaginal Fluid

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To determine whether bacterial vaginosis (BV), also known as nonspecific vaginitis, could be diagnosed by evaluating a Gram stain of vaginal fluid, we examined samples from 60 women of whom 25 had clinical evidence of BV and 35 had candidal vaginitis or normal examinations. An inverse relationship between the quantity of the *Lactobacillus* morphotype (large gram-positive rods) and of the *Gardnerella* morphotype (small gram-variable rods) was noted on Gram stain ($P < 0.001$). When Gram stain showed a predominance (3 to 4+) of the *Lactobacillus* morphotype with or without the *Gardnerella* morphotype, it was interpreted as normal. When Gram stain showed mixed flora consisting of gram-positive, gram-negative, or gram-variable bacteria and the *Lactobacillus* morphotype was decreased or absent (0 to 2+), the Gram stain was interpreted as consistent with BV. Gram stain was consistent with BV in 25 of 25 women given a clinical diagnosis of BV and in none of 35 women with candidal vaginitis or normal examinations. Duplicate slides prepared from 20 additional specimens of vaginal fluid were stained by two methods and examined by three evaluators. Interevaluator interpretations and intraevaluator interpretations of duplicate slides were in agreement with one another and with the clinical diagnosis ≥90% of the time. We concluded that a microscopically detectable change in vaginal microflora from the *Lactobacillus* morphotype, with or without the *Gardnerella* morphotype (normal), to a mixed flora with few or no *Lactobacillus* morphotypes (BV) can be used in the diagnosis of BV.

A clinical diagnosis of nonspecific vaginitis can be based on the presence of a characteristic homogeneous grey discharge, a vaginal fluid pH of >4.5, a positive amine odor test, and the identification of "clue cells" by microscopic examination of vaginal fluid mixed with saline (1a, 16). Vaginal cultures may be obtained to exclude yeast and *Trichomonas vaginalis*, and endocervical cultures may be obtained to exclude *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. There are no commonly available tests that enable the clinical microbiologist to aid the clinician in diagnosing nonspecific vaginitis. *Gardnerella vaginalis* is almost universally found in high concentration in the vaginal fluid of women with nonspecific vaginitis, but because it is often found in the vaginal flora of normal women, the significance of a positive vaginal culture for this organism in an individual patient is uncertain (14, 18, 24), even when semiquantitative cultures are done. Anaerobic bacteria have also been associated with nonspecific vaginitis (16, 23). As with *G. vaginalis*, interpretation depends upon quantitative counts and is not recommended for the clinical laboratory. Gas-liquid chromatography (GLC) for the detection of bacterial organic acid metabolites (23) and thin-layer chromatography for the detection of diamines (4) show patterns characteristic of nonspecific vaginitis, but the equipment for these tests is unavailable to many laboratories. We will use the term bacterial vaginosis (BV) to refer to the entity because of its association with bacteria rather than fungi or protozoa, because no single bacterial agent can be regarded as solely responsible for the syndrome, and because of the absence of a true inflammatory response in most cases (10).

The specific vaginitides caused by *T. vaginalis* and candida are most commonly diagnosed by microscopic examination of vaginal fluid. Microscopy has also been used for the diagnosis of BV. Gardner and Dukes reported that the appearance of clue cells (i.e., vaginal epithelial cells studded with coccobacillary organisms) in vaginal fluid wet mounts was diagnostic for

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*Haemophilus vaginalis* vaginitis (7, 8). However, Smith et al. (21) and Akerlund and Mardh (1) subsequently reported that the presence of clue cells on Gram-stained vaginal smears and cervical Papanicolaou smears was not useful for the diagnosis of BV. Gardner and Dukes (7, 8) and Dunkelberg (6) described a Gram stain appearance which was characteristic of BV. Normal vaginal fluid contained only *Lactobacillus* morphotypes, whereas fluid from BV patients had many small gram-negative organisms resembling *G. vaginalis* in the absence of *Lactobacillus* morphotypes. In a recent publication, Balsdon et al. (2) again noted a characteristic microscopical appearance of vaginal discharge from patients with BV.

Although the literature suggests that a Gram stain may be used for the diagnosis of BV, this method has not been formally compared with clinical, microbiological, or biochemical criteria for the diagnosis of BV and is not currently being used by most clinicians or laboratorians. We have already shown that the bacterial vaginal flora demonstrable by vaginal fluid culture from BV patients differs from that demonstrable by vaginal fluid culture in healthy patients (23). The purpose of this study was to see whether these differences were also evident by direct Gram stain of vaginal fluid, to correlate our Gram stain interpretation with the results of clinical examination and with the isolation of *G. vaginalis*, and to reexamine the usefulness of this method for the diagnosis of BV.

## MATERIALS AND METHODS

**Clinical examination.** The patient population was drawn from 397 consecutive women who attended the Women's Clinic in the student health center at the University of Washington. All patients were examined by one of us (R.A.) who selected for the review of Gram stains an arbitrary but representative subset of 60 women representing various diagnoses. The subset included patients given clinical diagnoses of BV alone (21 cases), BV plus trichomonal vaginitis (2 cases), BV plus candidal vaginitis (2 cases), candidal vaginitis (10 cases), and a normal diagnosis (25 cases). A BV diagnosis was made when three of the following four characteristics were detected: vaginal pH of >4.5, thin homogeneous discharge, clue cells, and "fishy" amine odor after the addition of 10% potassium hydroxide. The examination was considered normal when three of these criteria were not met and neither fungi nor trichomonads were detected microscopically. The microscopic detection of fungal elements or motile trichomonads was considered diagnostic for yeast or *T. vaginalis* vaginitis, respectively. An evaluation of this method for the diagnosis of BV and a description of the whole study population may be found elsewhere (1a).

**Microbiological examination.** The clinical examination included a culture for *G. vaginalis* (*H. vaginalis*). A cotton-tipped applicator was used to transfer vaginal fluid onto a human blood bilayer medium (HB medium) (24). HB plates were examined for *G. vaginalis* after 48 and 72 h of incubation at 37°C in 5% $CO_2$ in air. *G. vaginalis* colonies appeared as small beta-hemolytic colonies on HB agar. Growth was quantitated as follows: 1+, <10 colonies in the first inoculation zone; 2+, >10 colonies in the first zone and <10 colonies in the second zone; 3+, >10 colonies in the second zone and <10 colonies in the third zone; 4+, >10 colonies in the fourth zone. The identification was confirmed by their characteristic Gram stain morphology showing small pleomorphic gram-variable rods, fermentation of starch and glucose but not mannitol, the inability to produce green discoloration of chocolate agar, and the inability to produce catalase and oxidase.

The fermentation medium used for identification of *G. vaginalis* consisted of 1% Proteose Peptone no. 3 (Difco Laboratories), 0.3% meat extract (BBL Microbiology Systems), 0.5% NaCl, and 1% Andrade indicator. The pH was adjusted to 7.1 before the medium was autoclaved. To this base the appropriate sugar (1%) and fetal calf serum (1%) were added.

**Gram stains.** During the clinical examination, a direct smear was prepared by transferring vaginal fluid to a glass microscope slide with a cotton-tipped applicator stick. The slides were labeled with only the date of collection and the patient's study number and initials and were then air dried and stored in the dark.

R.A. selected 60 slides to represent the various clinical diagnoses. In the laboratory, the smears were heat fixed and stained by the Kopeloff modification of the Gram stain (9) and using basic fuchsin as the counterstain. This will be referred to as the VPI (Virginia Polytechnic Institute) method. All of the stains were interpreted by C.A.S. without knowledge of the clinical or microbiological findings. Each microbial morphotype was quantitated under oil immersion (×1,000) by the following scheme: 1+, <1 per field; 2+, 1 to 5 per field; 3+, 6 to 30 per field; 4+, >30 per field. Large gram-positive bacilli were assumed to be the *Lactobacillus* morphotype. Smaller gram-variable bacilli were assumed to be the *Gardnerella* morphotype. Other organisms were categorized by morphology only, e.g., gram-negative bacilli, curved rods, gram-positive cocci in chains, and fusiforms.

When the *Lactobacillus* morphotype was present alone or in combination only with the *Gardnerella* morphotype, the smear was interpreted as normal. When a more mixed flora, including not only the *Gardnerella* morphotype but also other gram-negative and gram-positive bacteria, such as curved rods, gram-negative rods, fusiforms, and gram-positive cocci, was present and when the *Lactobacillus* morphotype was absent or present only in low numbers (1 to 2+), the smear was interpreted as consistent with BV. After all of the Gram stain smears had been evaluated and the Gram stain diagnoses were made, the results were compared with those of the clinical and microbiological examinations.

To examine interevaluator and intraevaluator variability and to evaluate the influence of the Gram stain method on stain interpretation, an additional 20 consecutive vaginal wash specimens received in the laboratory were stained by two methods and were examined by three individuals. The method for the collection of vaginal wash specimens has been described previously (23). Duplicate slides were pre-

pared by spreading a loopful of fluid on each of two glass slides, which were allowed to air dry. One set of slides was stained by the VPI method, and the other set was stained by the Gram stain method described in the *Manual of Clinical Microbiology* (13), which will be referred to as the MCM method. Each set of slides was examined independently by three individuals, and a Gram stain diagnosis was made by the criteria given above. The results were subsequently compared with the clinical diagnosis.

GLC of vaginal fluid was performed as previously reported (23) by the methods described in the *Anaerobe Laboratory Manual* (9). GLC was defined as abnormal when the S/L ratio (succinate peak height in millimeters/lactate peak height in millimeters) was ≥0.4, the acetate peak height was >2 mm, or propionate or butyrate was detected.

**Statistical methods.** Data were evaluated by the chi-square and Fisher exact tests.

## RESULTS

**Gram stain patterns representative of BV or normal flora.** Examples of Gram-stained smears of vaginal fluid are shown in Fig. 1. In Fig. 1A, only large gram-positive bacilli are evident. This is the typical appearance of the organisms identified as having the *Lactobacillus* morphotype on Gram stain. This patient had a normal clinical examination and a normal GLC pattern, and no *G. vaginalis* organisms were isolated from the vagina. Normal vaginal epithelial cells are also evident. In Fig. 1B, two bacterial morphotypes are evident, large gram-positive bacilli (the *Lactobacillus* morphotype) and smaller coccobacillary gram-positive organisms. These latter organisms were identified as consistent with the *Gardnerella* morphotype. Stains such as this one, showing the presence of both *Lactobacillus* and *Gardnerella* morphotypes, were interpreted as normal. This patient had 4+ growth of *G. vaginalis*, a normal GLC pattern, and no clinical evidence of BV. Figure 1C shows a smear interpreted as consistent with BV. The flora is composed mainly of small gram-positive organisms of the *Gardnerella* morphotype, gram-

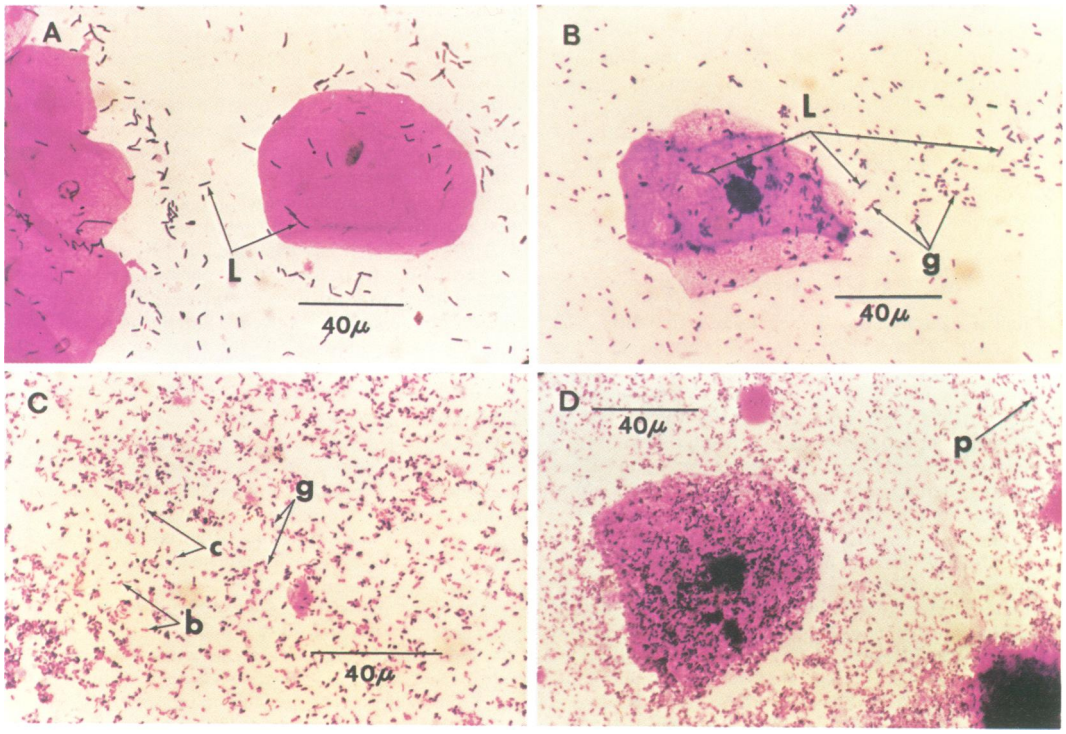


FIG. 1. Vaginal fluid smears stained by the Kopeloff modification of the Gram stain (VPI method). L, *Lactobacillus* morphotype; g, *Gardnerella* morphotype; p, gram-positive cocci; b, small gram-negative rods; c, curved rods. The original magnification is given. (A) Vaginal squamous epithelial cells and 4+ large gram-positive rods (*Lactobacillus* morphotype). No *G. vaginalis* was isolated. Clinical examination was normal. ×800. (B) Vaginal squamous epithelial cell, 3+ large gram-positive rods (*Lactobacillus* morphotype), and 4+ small gram-positive rods (*Gardnerella* morphotype). 4+ *G. vaginalis* was isolated. Clinical examination was normal. ×800. (C) Mixed flora including 3+ small gram-negative rods, 4+ *Gardnerella* morphotype, and 4+ curved rods. No *Lactobacillus* morphotype was present. Clinical diagnosis of BV. 4+ *G. vaginalis* was isolated. ×1,000. (D) Clue cell and mixed flora, including 1+ gram-positive cocci from the same specimen as Fig. 1C. ×800.

TABLE 1. Vaginal microflora in patients with and without BV as determined by Gram-stained smear of vaginal fluid

| Morphotype of organisms seen on Gram-stained smear | Clinical diagnosis | | P value |
|---|---|---|---|
| | With BV ($n$ = 25) | Without BV ($n$ = 35) | |
| Gram-positive cocci | 15 | 3 | <0.001 |
| Gram-negative bacilli | 24 | 0 | <0.001 |
| *Lactobacillus* morphotype (0–2+) | 25 | 5 | <0.001 |
| *Gardnerella* morphotype | 25 | 9 | <0.001 |
| Curved rods | 11 | 0 | <0.001 |

negative coccobacilli, and curved rods. No cells resembling the *Lactobacillus* morphotype are seen. Clue cells were also present (Fig. 1D). This patient had a clinical diagnosis of BV, 4+ growth of *G. vaginalis*, and an abnormal GLC pattern.

**Correlation of Gram stain pattern with clinical diagnosis.** The Gram stain diagnosis was interpreted as consistent with BV in 25 of 25 women given a clinical diagnosis of BV and in none of 35 women not given a clinical diagnosis of BV. The organisms seen in smears of vaginal fluid from patients with and without a clinical diagnosis of BV are given in Table 1. Gram-positive cocci were seen in 15 of 25 patients with BV and in 3 of 35 patients without BV ($P < 0.001$). Curved rods were seen in 11 of 25 patients with BV and in none of 35 patients without BV ($P < 0.001$). The *Gardnerella* morphotype was seen in 25 of 25 patients with BV and in 9 of 35 patients without BV ($P < 0.001$). Small gram-negative bacilli resembling *Bacteroides* spp. were seen in 24 of 25 patients with BV and in none of 35 patients without BV ($P < 0.001$). The *Lactobacillus* morphotype was absent or present only in low quantities (1 to 2+) in 25 of 25 patients with BV and in 5 of 35 patients without BV ($P < 0.001$).

The semiquantitative assessment of *Gardnerella* morphotypes observed on the Gram stain is highly correlated (Table 2) with the semiquantitative assessment of *G. vaginalis* growth by culture ($\chi^2_4 = 35.8$; $P < 0.0001$). Gram stain and culture were both positive or both negative in 49 of 60 cases ($P < 0.001$). In the nine cases in which the culture was positive and the Gram stain was negative, four had 2+ growth of *G. vaginalis* and five had 3+ growth. In the 34 instances in which both the culture and the Gram stain were positive for *G. vaginalis*, 11 had 3+ growth and 23 had 4+ growth. There were two cases in which the Gram stain was positive and the culture was negative.

Table 3 shows the strong inverse relationship between the quantity of *Lactobacillus* and *Gardnerella* morphotypes seen in the 60 Gram-stained smears ($\chi^2_4 = 39.74$; $P < 0.0001$) and the clinical diagnosis. When the *Lactobacillus* morphotype was scored as 4+, the *Gardnerella* morphotype was usually absent. When the *Gardnerella* morphotype was scored as 3+ or 4+, the quantity of the *Lactobacillus* morphotype was generally diminished. Of the 29 patients who had 2 to 4+ *Gardnerella* and 0 to 2+ *Lactobacillus* morphotypes, 25 had a clinical diagnosis of BV. Of the other 31 patients, none had BV and 30 had 3 to 4+ *Lactobacillus* morphotype.

Vaginal fluid from 29 of the 60 patients was examined by GLC. Of these 29, 10 had both a Gram stain diagnosis and a clinical diagnosis of

TABLE 2. Comparison of semiquantitative isolation of *G. vaginalis* on HB medium versus semiquantitative counts of *Gardnerella* morphotype on Gram-stained smears of vaginal fluid

| Quantity of *Gardnerella* morphotype on Gram stain[a] | Quantity of *G. vaginalis* on culture[a] | | | | |
|---|---|---|---|---|---|
| | 0+ | 1+ | 2+ | 3+ | 4+ |
| 0+ | 15 | 0 | 4 | 5 | 0 |
| 1+ | 0 | 0 | 0 | 0 | 0 |
| 2+ | 1 | 0 | 0 | 1 | 1 |
| 3+ | 0 | 0 | 0 | 4 | 0 |
| 4+ | 1 | 0 | 0 | 6 | 22 |

[a] The quantity of *G. vaginalis* on culture and on Gram stain was classified for analysis as 0+, 1 to 2+, and 3 to 4+ due to small cell frequencies. $\chi^2_4 = 35.76$; $P < 0.0001$.

TABLE 3. Clinical diagnosis of BV in relation to Gram stain quantitation of *Lactobacillus* and *Gardnerella* morphotypes[a]

| Quantity of *Lactobacillus* morphotype[b] | Quantity of *Gardnerella* morphotype[b] | | | | |
|---|---|---|---|---|---|
| | 0+ | 1+ | 2+ | 3+ | 4+ |
| 0+ | 0 | 0 | 1 (1)[c] | 0 | 15 (13) |
| 1+ | 0 | 0 | 1 (1) | 0 | 8 (7) |
| 2+ | 1 | 0 | 0 | 1 | 3 (3) |
| 3+ | 1 | 0 | 0 | 1 | 0 |
| 4+ | 24 | 0 | 1 | 1 | 2 |

[a] The quantity of *Gardnerella* and *Lactobacillus* morphotypes was classified for analysis as 0+, 1 to 2+, and 3 to 4+ due to small cell frequencies. $\chi^2_4 = 39.74$; $P < 0.0001$.

[b] See text for method of quantitation.

[c] The number in parentheses indicates the number of patients with BV. There were four stains which had more *Gardnerella* than *Lactobacillus* morphotypes but were not consistent with BV because the other mixed coccobacillary flora was not present. These four did not have a clinical diagnosis of BV.

BV. The GLC was abnormal in all 10 of these cases. There was an additional abnormal GLC from a patient who had both a Gram stain and a clinical diagnosis of candidal vaginitis. The remaining 18 patients had a normal GLC pattern and no clinical evidence of BV.

The Gram stain was also of some value in the diagnosis of specific vaginitides. Of the 60 patients, 4 had multiple infections. In the two patients who had both BV and *T. vaginalis* vaginitis, trichomonads were detected on Gram stain (22). In two patients who had both BV and candidal vaginitis, only BV was diagnosed by Gram stain. Four of 10 patients with a clinical diagnosis of candidal vaginitis alone and 2 of 25 who had a normal clinical exam had fungal elements on Gram stain.

**Interevaluator and intraevaluator reproducibility of BV diagnosis by Gram stain.** Twenty additional specimens from 10 subsequent patients were stained by both the VPI and MCM methods and were examined by three individuals (Table 4). The clinical diagnoses were BV in 6, candidal vaginitis in 1, and normal in 13 cases. Overall, the Gram stain diagnosis agreed with the clinical diagnosis ≥90% of the time, as did interpretations made by the same individual of specimens stained by the two methods. Evaluator 1 interpreted the MCM stain as BV and the VPI stain as trichomonal vaginitis in one patient given a clinical diagnosis of BV, and the MCM stain was interpreted as normal and the VPI stain as BV in one normal patient. Evaluator 2 felt that two specimens (one with a normal exam and one with BV) could not be evaluated because of insufficient material. She interpreted the MCM and the VPI stains as BV in one normal patient. The interpretations of evaluator 3 agreed with one another and with the clinical exam in all instances. She noted the presence of epithelial cells with attached *Lactobacillus* morphotypes in the normal specimen interpreted as BV by evaluators 1 and 2, both of whom noted clue cells in the specimen.

TABLE 4. Accuracy and reproducibility of Gram stain diagnosis of BV: agreement between duplicate interpretations and clinical diagnosis by three evaluators[a]

| Evaluator no. | No. of pairs in agreement/no. of pairs interpreted | |
|---|---|---|
| | Intraevaluator agreement in interpretation of 20 specimens stained by two methods | Agreement between Gram stain and clinical diagnosis |
| 1 | 18/20 | 18/20 |
| 2 | 18/18 | 17/18 |
| 3 | 20/20 | 20/20 |

[a] See text for explanation of discrepancies.

**Clue cells and amine odor.** Epithelial cells resembling clue cells were seen on direct Gram stains, but their presence or absence was difficult to evaluate by Gram stain of these heavily smeared slides. Wet mounts were examined for the presence of clue cells during the clinical examination. Clue cells were present in 30 of 31 specimens from patients with BV and in none of 49 specimens from patients without a clinical diagnosis of BV ($P < 0.0001$).

The amine odor test was positive in 28 of 31 specimens from patients with BV and in none of 49 patients without a clinical diagnosis of BV ($P < 0.0001$).

## DISCUSSION

Direct microscopic examination of clinical material is often used in the diagnosis of bacterial infections. In this paper, we have evaluated the Gram stain method for the diagnosis of BV in patients evaluated by standard clinical and microbiological criteria.

When the *Lactobacillus* morphotype (large gram-positive rods) was present alone or in combination only with the *Gardnerella* morphotype (small gram-variable rods), the smear was interpreted as normal. When the *Lactobacillus* morphotype was absent or present in low numbers (1 to 2+) and the *Gardnerella* morphotype and other forms predominated, the smear was interpreted as consistent with BV. All 25 of the cases diagnosed as BV by clinical examination were also diagnosed as BV by Gram stain. The Gram stain technique did not allow distinction between symptomatic and asymptomatic patients; 12 of the 25 patients with BV reported no symptoms.

The increased prevalence of gram-negative rods, gram-positive cocci, and other organisms seen on the smears from BV patients is consistent with the previously reported increase in the prevalence and quantity of *Bacteroides* spp. and butyrate-producing *Peptococcus* spp. and an increase in their metabolic products in vaginal fluid from women with BV (23). The decrease in the prevalence and concentration of the *Lactobacillus* morphotype on Gram stain in women with BV is paralleled by a decrease in the quantity and prevalence of cultivable *Lactobacillus* morphotype and a decrease in lactic acid in vaginal fluid in patients with BV (23, 24). The presence of curved rods also was correlated with the diagnosis of BV. Motile curved rods have been noted by other investigators (17), but the identity of these organisms and their role in BV is not clear. Although vaginal fluids from patients with nonspecific vaginitis have previously been described as yielding pure cultures of *G. vaginalis* (6–8), such specimens actually contain a mixture of gram-variable *G. vaginalis* and

anaerobes (16), including *Bacteroides* spp., *Peptococcus* spp., curved rods (23), and *Eubacterium* spp. (C. A. Spiegel, P. Davick, P. A. Totten, K. C. S. Chen, D. A. Eschenbach, R. Amsel, and K. K. Holmes, Scand. J. Infect. Dis., in press).

Examination of Gram-stained smears of vaginal fluid is a less sensitive technique than culture for the detection of vaginal colonization by *G. vaginalis*. None of the nine specimens which had a negative stain and a positive culture had >3+ quantity of *G. vaginalis* on culture. Perhaps the two specimens which had a positive stain and a negative culture had anaerobic strains of *G. vaginalis* (15). The detection of *G. vaginalis* either by Gram stain or by culture cannot be recommended as a method for the diagnosis of BV because it is often a member of the normal vaginal flora. This lack of value of a positive vaginal culture for *G. vaginalis* as a tool in the diagnosis of BV has been reported (1a, 24), but it deserves reemphasis because of the frequency of clinical requests for *G. vaginalis* isolation.

Ison et al. (12) recently used methods similar to ours to compare culture and microscopy for the detection of *G. vaginalis* in vaginal fluid. In contrast with our results, however, they did not find a correlation between the microscopic and cultural methods. The *G. vaginalis* culture was positive in 25 (80%) of 31 specimens with and 20 (65%) of 31 specimens without microscopically detectable *G. vaginalis*. The larger number of microscopic false-negative tests may have been due in part to differences in methodology. Ison et al. prepared slides from vaginal fluid diluted in saline and examined them for the presence of large amounts of gram-variable rods, whereas we prepared slides with undiluted vaginal fluid and examined them for the presence of any small gram-variable rods.

Gram stains were insensitive for the diagnosis of yeast vaginitis even when compared with wet mounts, perhaps because the smears were quite thick, having been prepared from undiluted vaginal fluid.

There is an inverse relationship between the presence or absence and concentration of *Gardnerella* and *Lactobacillus* morphotypes in the Gram-stained smears. This observation has also been made in culture studies (23, 24). The significance of this phenomenon in the pathogenesis of BV is currently under investigation.

When the criteria described here were used to differentiate patients with BV from normal controls and when duplicate smears prepared by two different methods were interpreted by each evaluator, the results were reproducible among three evaluators. The few discrepancies which occurred appeared to be due to the presence of epithelial cells with adherent *Lactobacillus* morphotypes which, on low-power examination, were interpreted as clue cells.

The presence of clue cells detected in a wet preparation of vaginal fluid also correlated very well with a clinical diagnosis of BV. This is not surprising, since the presence of clue cells was one of the four criteria used to define BV clinically in this study.

Attempts to characterize vaginal health by microscopy have appeared in the literature for years (11, 20, 21, 25). Doderlein (cited in reference 8) described three grades of vaginal cleanliness that he correlated with vaginal health. Subsequent studies have shown that these criteria are inadequate for the diagnosis of vaginitis (11, 25). More recent data, including those presented here, help to explain some of the discrepancies. Grade I, which indicates a clean vagina, allows for the presence of yeast and so combines normal women and those with yeast vaginitis. Grade II, intermediate between a clean vagina and Doderlein's pathological flora, spans a wide pH range and is associated with a mixed vaginal flora. Included in this group might be samples we would classify as normal in the presence of *Lactobacillus* and *Gardnerella* morphotypes. Such normal samples will have an elevated pH when contaminated with menstrual blood. Grade II also includes samples we would classify as BV with 1 to 2+ *Lactobacillus* morphotypes. Grade III, Doderlein's pathological flora, lacks the *Lactobacillus* morphotype, a characteristic of many of our BV patients. From the description of the discharge, "profuse and purulent or rather scanty and watery," this group appears to be a combination of patients with trichomoniasis or BV, respectively. Weinstein (25) and Hunter and Long (11) related these grades to culture results and found no correlation. However, these studies were performed before the improvements in anaerobic culture techniques and before *G. vaginalis* was described. The diagnosis of vaginitis was based on the presence of symptoms so that the presence of grade III flora in an asymptomatic patient was considered a contradiction.

It is interesting to note that observations made in these previous publications were often subsequently ignored. Although coliforms are not usually recovered from vaginal fluid, they were often noted on Gram stains. Perhaps the organisms described as coliforms in older studies were *Bacteroides* spp. Hunter and Long (11) noted "extreme pleomorphism and bizarre cultural appearances, not only of the lactobacilli but also of the organisms classified as diphtheroids," but they believed them all to be *Lactobacillus* morphotypes. They also described streptococci which grew slowly, produced a

narrow zone of hemolysis, and gave an irregular Gram stain. These organisms may have been *G. vaginalis*. The description of cocci-dominated vaginitis by Bergman et al. (3) and micrographs of samples from *Kokken kolpitis* (coccal vaginitis) described by Schnell and Meinrinken (19) are consistent with our criteria for BV. Curved rods have long been associated with vaginal discharge (5, 20), but their role in BV is less well studied.

This study was not the first attempt to diagnose vaginitis by microscopic examination of vaginal fluid, but rather a reevaluation of the method by using the new, more precise and objective criteria for making a clinical diagnosis, an improved method for *G. vaginalis* isolation, and an increased knowledge about normal and pathological flora of the vagina. In so doing, we have helped explain why microscopic methods did not correlate well with the clinical and microbiological data collected in some past studies.

The current method for the diagnosis of BV includes observation of the appearance of the vaginal fluid, determination of pH, detection of an amine-like odor, and microscopic examination of a wet mount of vaginal fluid. Because the necessary equipment and expertise are not always available to clinicians, the availability of laboratory methods for the diagnosis of BV would be valuable. A specimen for GLC or thin-layer chromatography is appropriate, but not all laboratories have the equipment to do these tests. The microscopic methods detailed here for the diagnosis of vaginosis would fit well into a clinical microbiology setting and could be used to complement or confirm the clinician's evaluation of the patient with abnormal vaginal discharge. It could be argued that direct microscopic examination of a wet preparation of vaginal fluid should be done to rule out *T. vaginalis* in any patient with vaginal discharge and that the presence of clue cells can also be noted in the examination. However, these examinations are often performed in clinics by individuals with varying skills because immediate diagnosis is desired or because transport of freshly obtained vaginal fluid to the laboratory is inconvenient or impossible. In such cases, availability of a permanent smear for laboratory confirmation of diagnosis is desirable. In other cases, evaluation of wet preparations is not convenient in either the clinic setting or the laboratory. The Gram-stained smear method described here should make the diagnosis of BV easier for clinicans and laboratorians.

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## LITERATURE CITED

1. **Akerlund, M., and P. A. Mardh.** 1974. Isolation and identification of *Corynebacterium vaginale (Haemophilus vaginalis)* in women with infections of the lower genital tract. Acta Obstet. Gynecol. Scand. **53**:85–90.

1a. **Amsel, R., P. A. Totten, C. A. Spiegel, K. C. S. Chen, D. Eschenbach, and K. K. Holmes.** 1983. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiological associations. Am. J. Med. **74**:14–22.

2. **Balsdon, M. J., G. E. Taylor, L. Pead, and R. Maskell.** 1980. *Corynebacterium vaginale* and vaginitis: a controlled trial of treatment. Lancet **i**:501–504.

3. **Bergman, S., K.-M. Lundgren, and P. Lundstrom.** 1965. *Haemophilus vaginalis* in vaginitis. Acta Obstet. Gynecol. Scand. **44**:8–17.

4. **Chen, K. C. S., R. Amsel, D. A. Eschenbach, and K. K. Holmes.** 1982. Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. J. Infect. Dis. **145**:337–345.

5. **Cruickshank, R., and A. Sharman.** 1934. The biology of the vagina in the human subject. J. Obstet. Gynaecol. Br. Emp. **41**:208–226.

6. **Dunkelberg, W. E.** 1965. Diagnosis of *Haemophilus vaginalis* vaginitis by gram-stained smears. Am. J. Obstet. Gynecol. **91**:998–1000.

7. **Gardner, H. L., and C. D. Dukes.** 1955. *Hemophilus vaginalis* vaginitis. Am. J. Obstet. Gynecol. **69**:962–976.

8. **Gardner, H. L., and C. D. Dukes.** 1959. *Hemophilus vaginalis* vaginitis. Ann. N.Y. Acad. Sci. **83**:280–289.

9. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.

10. **Holmes, K. K., C. Spiegel, R. Amsel, D. A. Eschenbach, K. C. S. Chen, and P. Totten.** 1981. Nonspecific vaginosis. Scand. J. Infect. Dis. **26**:110–114.

11. **Hunter, C. A., and K. R. Long.** 1958. A study of the microbiological flora of the human vagina. Am. J. Obstet. Gynecol. **75**:865–871.

12. **Ison, C. A., S. G. Dawson, J. Hilton, G. W. Csonka, and C. S. F. Easmon.** 1982. Comparison of culture and microscopy in the diagnosis of *Gardnerella vaginalis* infection. J. Clin. Pathol. **35**:550–554.

13. **Lennette, E. H., A. Ballows, W. J. Hausler, and J. P. Truant (ed.).** 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.

14. **Malone, B. H., M. Schreiber, N. J. Schneider, and L. V. Holdeman.** 1975. Obligately anaerobic strains of *Corynebacterium vaginale (Haemophilus vaginalis)*. J. Clin. Microbiol. **2**:272–275.

15. **McCormack, W. M., J. R. Evrard, C. F. Laughlin, B. Rosner, S. Alpert, V. A. Crockett, D. McComb, and S. H. Zinner.** 1981. Sexually transmitted conditions among women college students. Am. J. Obstet. Gynecol. **139**:130–133.

16. **Pheifer, T. A., P. S. Forsyth, M. A. Durfee, H. M. Pollock, and K. K. Holmes.** 1978. Nonspecific vaginitis: role of *Haemophilus vaginalis* and treatment with metronidazole. N. Engl. J. Med. **298**:1429–1434.

17. **Popp, W.** 1977. The diagnosis and treatment of mixed anaerobic vaginal discharges. Geburtshelfe Frauenheilkd. **37**:432–437.

18. **Sautter, R. L., and W. J. Brown.** 1980. Sequential vaginal cultures from normal young women. J. Clin. Microbiol. **22**:479–484.

19. **Schnell, J. D., and H. Meinrinken.** 1973. Zytologie und

Mikrobiologie der Vagina. Wissenschafts-Verlag, Köln.
20. **Schröder, R.** 1921. Zur Pathogenese und Klinik des vaginalen Fluors. Zentrabl. Gynak. **45**:1350–1361.
21. **Smith, R. F., H. A. Rodgers, P. A. Hines, and R. M. Ray.** 1977. Comparisons between direct microscopic and cultural methods for recognition of *Corynebacterium vaginale* in women with vaginitis. J. Clin. Microbiol. **5**:268–272.
22. **Sobrepeña, R. L.** 1980. Identification of *Trichomonas vaginalis* in Gram-stained smears. Lab. Med. **11**:558–560.
23. **Spiegel, C. A., R. Amsel, D. Eschenbach, F. Schoenknecht, and K. K. Holmes.** 1980. Anaerobic bacteria in nonspecific vaginitis. N. Engl. J. Med. **303**:601–607.
24. **Totten, P. A., R. Amsel, J. Hale, P. Piot, and K. K. Holmes.** 1982. Selective differential human blood bilayer media for isolation of *Gardnerella (Haemophilus) vaginalis*. J. Clin. Microbiol. **15**:141–147.
25. **Weinstein, L.** 1937. The bacterial flora of the human vagina. Yale J. Biol. Med. **10**:247–260.